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RESPONSES OF BROILERS TO DIFFERENT DIETARY CONCENTRATIONS OF ORGANIC AND INORGANIC IRON IN RELATION TO IMMUNE REACTIONS

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ABSTRACT

Trace elements such as Fe, Cu, Mn, and Zn are essential for the growth of broiler chicks, as they play a crucial role in various immunological reactions within the body. Iron, in particular, is a vital nutrient for rapidly growing organisms.

This study aimed to investigate the effects of organic and inorganic iron on specific immune reactions in broiler chickens. Over a period of 35 days, the birds were fed diets containing different doses of iron sulfate and iron methionine (60 ppm and 300 ppm). The study evaluated the impact of these minerals on the bactericidal activity of lysozyme in serum and the migration of lymphocytes.

Analysis of the data obtained from this research allows for the optimization of immunological reactivity in broiler chickens. Findings indicate that appropriate supplementation of iron can significantly enhance immune function, thereby improving overall health and growth performance in poultry.

Key words: organic and inorganic iron, broiler chickens, bactericidal activity, lysozyme, lymphocyte migration.

Introduction

Iron is a biogenic element of great significance, particularly for rapidly growing organisms. Depending on the species and age of the animals, hemoglobin contains between 0.33% and 0.345% iron. It is found in myoglobin as well as in various respiratory and antioxidant enzymes, such as cytochrome oxidases and catalases (Bou-Abdallah *et al.*, 2008). The discussion regarding the role of iron in the immune system and the impact of its deficiency on individuals' morbidity is ongoing.

Recent studies highlight that iron plays a crucial role in various immune functions, including the proliferation and differentiation of immune cells. Insufficient iron levels have been linked to impaired immune responses, increased susceptibility to infections, and prolonged recovery times. Research indicates that iron deficiency can lead to an increase in pro-inflammatory cytokines and disrupt the balance of T helper cell responses, particularly inhibiting the Th1 response (Camaschella, 2015; Kato *et al.*, 2020).

Research on the effects of organic and inorganic iron on the immune status of humans and animals remains relatively scarce. In this context, Milanovic *et al.* (2008) reported a stronger skin hypersensitivity reaction in chickens fed organic iron in their diet. Recent investigations have also shown that iron can influence the gut microbiome, which in turn affects the immune response, highlighting the importance of balanced dietary iron in overall health (Davis *et al.*, 2021).

Moreover, there is solid evidence of the impact of iron deficiency on the functional activity of immune-competent cells. Studies have demonstrated a reduction in the bactericidal activity of macrophages and a decrease in the myeloperoxidase activity of neutrophils (Spear and Sherman,

1992). Furthermore, a recent meta-analysis suggests that addressing iron deficiency can significantly improve immune responses, particularly in populations at risk (Khan *et al.*, 2022).

The objective of the present study is to investigate the effects of organic and inorganic iron on certain immune responses in broiler chickens. The study examines the influence of these minerals on the bactericidal and lysozyme activity of serum and on the migration of lymphocytes.

Materials and Methods

Iron Sources

- ***Iron methionine (FeMeth*)** – synthesized at the Department of Organic Chemistry, University of Chemical Technology and Metallurgy (UCTM) – Sofia; containing 13.3% iron and 34% methionine.
- ***Iron sulfate ($FeSO_4 \cdot 7H_2O$)*** – "Merck", Germany; containing 20% iron.

Experimental Animals

The experiment included 55 Ross-Ikov broiler chickens, male and female, 10 days old. The birds were divided into 5 groups of 11 chickens each and kept under identical housing and feeding conditions. During the 35-day experimental period the birds received:

- Group I: 60 ppm iron methionine
- Group II: 60 ppm iron sulfate
- Group III: 300 ppm iron methionine
- Group IV: 300 ppm iron sulfate
- Group V**:** Control (no iron supplementation)

Blood samples for immunological analyses were collected on days 0, 15, and 35.

Bactericidal activity of the blood sera was evaluated using a standardized assay based on the serum's ability to inhibit the growth of a reference bacterial culture. The method involves comparing viable bacterial counts in the presence and absence of serum over a defined incubation period, allowing quantification of the serum's innate antimicrobial capacity.

Lysozyme activity was quantified turbidimetrically by monitoring the lysis of *Micrococcus lysodeikticus* cells. The decrease in turbidity of the bacterial suspension was recorded spectrophotometrically at a wavelength of 450 nm**,** which is the standard analytical wavelength for this assay due to the strong light-scattering properties of the substrate. Measurements were taken over a defined time interval to determine the rate of enzymatic activity

Leukocyte Migration Inhibition Test

Lymphocyte suspensions were obtained using a density gradient separation. After triple washing, cells were counted in a Türk chamber and adjusted to a concentration of 6×10^7/ml. The test was performed according to Aleksandrov and Filchev (1989).

Statistical Analysis of the Data

Based on the individual results obtained from the separate experiments a statistical analysis was performed. The data were analyzed using the Student's t-test method.

Results

The bactericidal activity of blood serum is presented in Fig. 1.

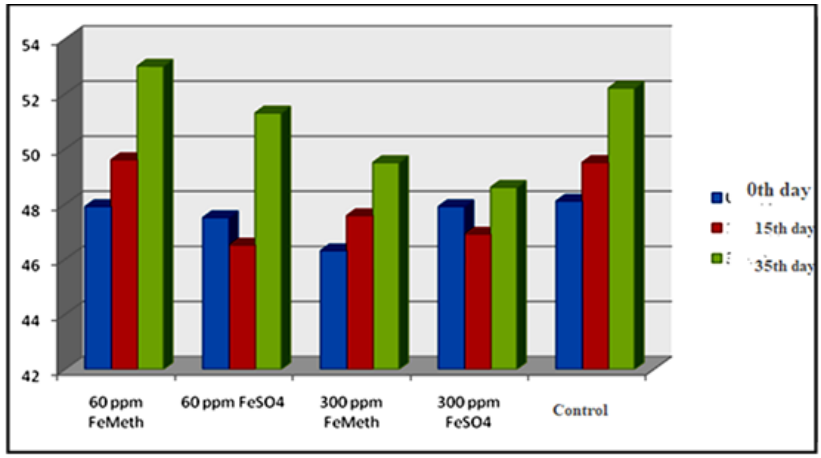

Figure 1: Dynamics of bactericidal activity of blood serum of chickrns treated with organic and inorganic iron

On day 15, chickens in group I (60 ppm FeMeth) showed a significant increase in bactericidal activity compared to the control group. By day 35, bactericidal activity in this group increased by 15.6%. In contrast, birds receiving 300 ppm of either iron source exhibited slight suppression of bactericidal activity.

These results indicate that the effect of iron supplementation on serum bactericidal activity is dose-dependent and influenced by the chemical form of iron.

Lysozyme activity results are shown in Fig. 2.

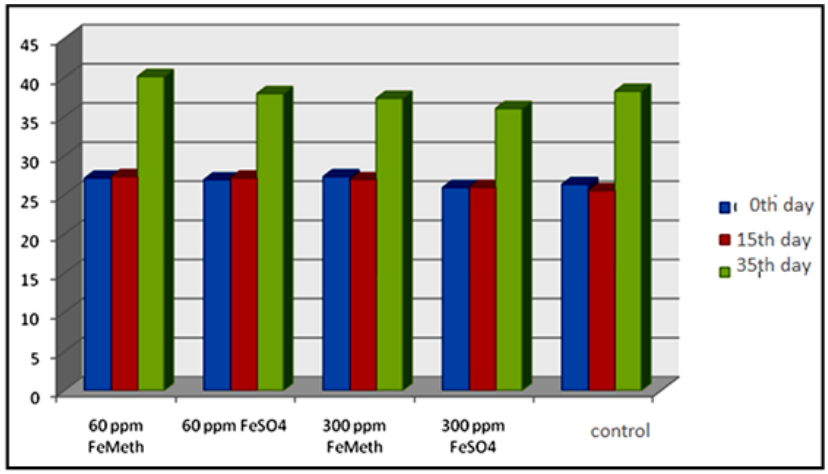

Figure 2: Dynamics of lysosomal activity

On day 15, no significant increase in lysozyme activity was observed in the experimental groups compared to controls. By day 35, a trend similar to that of bactericidal activity was evident: the highest lysozyme activity was recorded in the 60 ppm FeMeth group.

Lysozyme is a key component of innate immunity, stimulating phagocytosis and antibody synthesis, and contributing to the defense against bacterial infections.

The migration of blood lymphocytes is summarized in table 1.

The lowest inhibition of leukocyte migration was observed in group IV (300 ppm $FeSO_4$). These data indicate that both the form and concentration of iron influence leukocyte functionality.

Table 1 Migration of leukocytes from peripheral blood of chickens treated with organic and inorganic iron (60ppm and 300ppm) on day 35

GROUPS	Migration of leukocytes (mm^2)
I group (60 ppm FeMeth)	5,40 ± 0,83
II group (300 ppm FeMeth)	8,01±083
III group (60 ppm $FeSO_4$)	8.77±0.77
IV group (300 ppm $FeSO_4$)	13.30 ± 3,12
V -control	11,39 ± 0,91

Discussion

The results of the present study clearly demonstrate that both the form and dosage of dietary iron influence nonspecific immune responses in broiler chickens.

The increase in bactericidal and lysozyme activity observed in the 60 ppm FeMeth group is consistent with recent findings showing enhanced immune function following supplementation with organic iron chelates.

Chen *et al.* (2020) reported that organic iron improves antioxidant status and enhances innate immunity, increasing the activity of enzymes such as catalase and superoxide dismutase.

Shao *et al.* (2021**)** demonstrated higher macrophage activity and serum lysozyme levels in broilers fed organic iron.

These results support the conclusion that moderate supplementation with organic iron optimally stimulates innate immune mechanisms**.**

In contrast, high dietary iron doses (300 ppm) led to a mild suppression of bactericidal and lysozyme activity. This phenomenon is consistent with observations reported by Han *et al.* (2022)**,** who showed that excessive organic iron can induce oxidative stress and organ toxicity in broilers.

Leukocyte migration results in our study showed that Group IV (300 ppm $FeSO_4$) exhibited the least inhibition. This observation may reflect a compensatory adaptive response, although, to our knowledge, no published data currently confirm that high-dose inorganic iron enhances leukocyte motility. Moderate oxidative or metabolic stress can transiently influence leukocyte function, which could potentially explain this pattern.

In contrast, organic iron (Fe-Gly chelate) was generally more effective in modulating leukocyte functionality, consistent with previous studies demonstrating that supplementation with iron chelates significantly increased T-lymphocyte subsets ($CD4^+$ and $CD8^+$) and IL-2 production, indicating stimulation of cellular immune responses in broiler chickens (Jarosz *et al.*, 2016). These results suggest that organic iron forms may provide superior immunomodulatory effects compared to inorganic salts, although further research is needed to evaluate their specific impact on leukocyte migration.

Iron plays a dual role in immunity.

As a cofactor in respiratory and antioxidant enzymes, it supports phagocytosis, ROS production, and lysozyme activity**.**

Conversely, iron overload can increase free iron ions, generate ROS, damage cell membranes, and reduce immune cell viability.

Our data align with these mechanistic insights: low-dose organic iron (60 ppm FeMeth) enhances innate immunity, whereas higher doses risk oxidative stress and mild immunosuppression.

Recent studies on innovative iron forms, including nanoparticles, suggest potential benefits: Al-Nasser *et al.* (2024) demonstrated that magnetite iron nanoparticles combined with quercetin enhanced antioxidant defense, immune protection, and resistance to *Clostridium perfringens* infection in broilers. Jain *et al.* (2024) found that iron oxide nanoparticles improved jejunal morphology, serum metabolites, and overall growth performance.

These studies indicate that advanced forms of iron may optimize immune function and gut health**,** while avoiding the negative effects observed at high doses of conventional inorganic or organic iron.

Conclusion

The findings emphasize the importance of appropriate iron form and dose selection**:**

- Organic iron at moderate doses (60 ppm) can effectively enhance nonspecific immunity.
- Excessive iron, particularly inorganic forms, may suppress immunity and increase oxidative stress.
- Innovative iron formulations, such as nanoparticles or chelated forms, offer promising strategies for improving immune function without adverse effects.

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